TurboDDS Data Dependent Scanning for the Agilent 500 Ion Trap LC/MS

Data Sheet

TurboDDS is an advanced scanning form of the 500 Ion Trap LC/MS. TurboDDS can be used to automatically gain qualitative information based on simple operator-specified criteria. The system performs MS/MS and MS^n experiments in a single analytical run. The results assist analysts in structural elucidation and unknown identification.

Key Benefits Include:

- Data dependent scanning.
  Accelerate and simplify information captured from unknown samples.
- Extended mass range.
  Analyze large molecules by scanning from *m/z* 100 to 3500.
- High resolution scanning.
  Accurately determine charge state of multiply-charged ions—up to charge state 6.
- Rapid auto CID-scan.
  Optimize CID up to five times faster than existing instruments to provide more comprehensive identification of unknowns.
- Easy-to-use, data review software.
  Discover informative links between related data points. Generate user libraries effortlessly.
- Fast calculation.
  The Mass Ruler software tool measures the mass difference between peaks with two mouse clicks.
- Easy data export.
  Transfer results to third party databases (such as Mascot and Phenyx) to enhance information derived from the sample.

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Functionality of TurboDDS

The TurboDDS process starts with a "survey" or full scan. If the information content of this scan meets user-specified criteria, an MS^n process will be initiated automatically in the subsequent scan.

The MS/MS and MS^n data generated automatically during data dependent scanning are a key source of information for qualitative analysis. TurboDDS can be used for structural elucidation to identify true unknowns such as natural products or the metabolites of newly designed drugs.

Agilent MS Workstation has been extended with easy-to-use, data dependent scanning review software. It allows the examination of data in simple, yet insightful ways, linking related data points.

Precursor Ion Qualification – Trigger Conditions

Minimum intensity specification	Qualify ions from the survey scan by absolute (above a minimum threshold) and relative (minimum % ion ratio) intensity
Isotopic equivalency	Qualifies survey ions only if they are outside the specified isotopic equivalency window
Include list	Static include list, ordered by intensity or user-defined priority criteria When specified as an exclusive list, considers only user-selected ions for MS^n analysis
Exclude list	Static list eliminates ions on the list from consideration for data dependent scanning
Dynamic exclusions	Optionally applied after a specified number of scans are collected from the same precursor ion. Exclusion may be maintained for the entire run or lifted after a specified time elapsed
Charge state criteria	Precursor ions with a specified charge state can be excluded from the TurboDDS process

MS^n Parameters

Automatic MS^n	Automatic MS/MS or MS^n process on qualified precursor ions, often referred to as the "depth" of the analysis, where n specifies how many MS^n steps will be generated from one precursor ion. Maximum n = 5
Turbo CID	Automatic determination of optimum CID conditions by patented CID scanning
High-resolution scan of the precursor ion	Verifies charge state of the precursor ion
N number of precursor ions from a scan	Specifies the number of ions in a scan to be considered for additional MS/MS step(s). Often referred to as the "breadth" of the analysis. This parameter is set separately for ions in the survey scan and for ions from the MS/MS step. Maximum N = 10

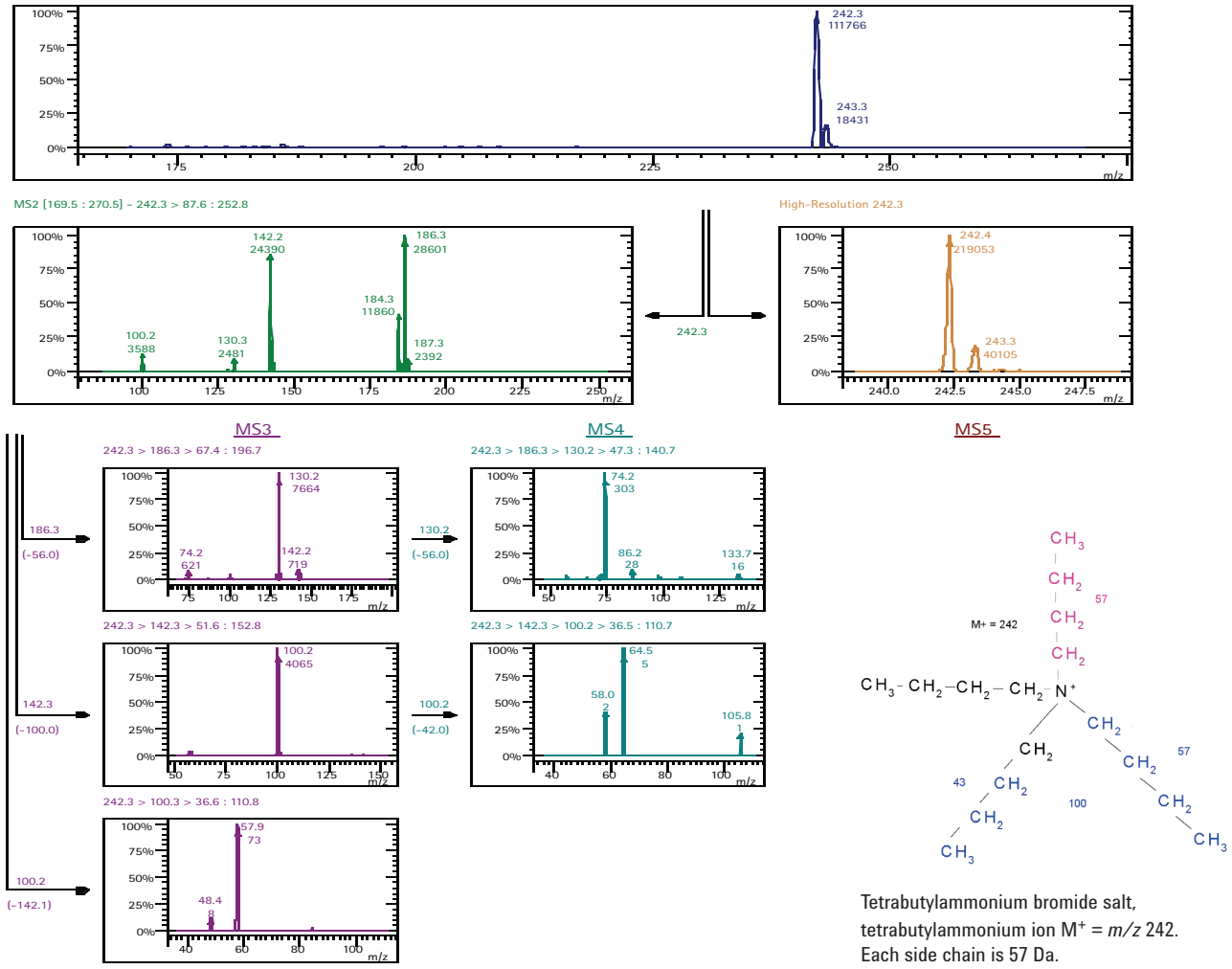


Figure 1. A simple structural elucidation with TurboDDS

Survey Scan

The survey scan is the starting point of the TurboDDS process. The tetra-n-butylammonium (TBA) ion was identified as the only target ion, *m/z* 242.3, to further analyze.

High Resolution Scan

The tree report shows the high resolution MS spectrum (in brown) of the analyte, confirming the single charge state for the target ion, *m/z* 242.3.

MS^2 Spectrum

From the survey scan, the precursor ion, *m/z* 242.3, is identified for MS/MS (MS^2) analysis. The MS^2 spectrum (in green) includes several product ions. The most abundant is *m/z* 186.3. This is TBA minus one butyl side chain (–56.0). The ion intensity values (displayed below the peak *m/z* values on the spectra) indicate that "Path A" is the predominant breakdown pathway.

MS^n Path "A"

From the MS/MS analysis the *m/z* 186.3 ion is isolated and the MS^3 analysis generates the product ion (*m/z* 130.2). This represents the loss of another butyl side chain group (–56.0). The *m/z* 130.2 ion is isolated and the MS^4 analysis produces a product ion (*m/z* 74.2), representing the loss of the third butyl side chain group (–56.0). An MS^5 analysis is not performed because the scan of the MS^4 product ions fails to meet the ion intensity threshold defined in the method.

MS^n Path "B"

The second most abundant product ion in the MS/MS spectrum is *m/z* 142.2. This is TBA minus one butyl side chain and one propyl group (–100.0). The *m/z* 142.2 ion is isolated and the MS^3 analysis produces a product ion (*m/z* 100.2). This represents the loss of another propyl group (–42.0). The *m/z* 100.2 ion is isolated and the MS^4 analysis creates a product ion (*m/z* 58.0), representing the loss of yet another propyl group (–42.0). The intensity of the MS^3 product ions meets the threshold requirement and the MS^4 analysis proceeds. However, the generated product ions have extremely low ion intensities - with limited significance.

MS^n Path "C"

The third most abundant product ion in the MS/MS spectrum is *m/z* 100.2. This is TBA minus one butyl side chain and two propyl groups (–142.0). The *m/z* 100.2 ion is isolated and the MS^3 analysis produces a product ion (*m/z* 57.9), representing the loss of yet another propyl group (–42.1). An MS^4 analysis is not performed because the intensity of the MS^3 product ions falls below the ion intensity threshold defined in the method.

Protein View

Match to: **AAN17824** Score: **223**
AF542068 NID: - Bos taurus
Found in search of Z:\Titan_LC-MS\R&D Chemist\SDS Training\SDS for MASCOT\S

Nominal mass (M_r): **69278**; Calculated pI value: **5.82**
NCBI BLAST search of AAN17824 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Bos taurus
Links to retrieve other entries containing this sequence from NCBI Entrez:
CAA41735 from Bos taurus
CAA76847 from Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **41%**

Matched peptides shown in **Bold Red**

```
     1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
    51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
   101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
   151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
   201 LLPKIETMRE KVLTSSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
   251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
   301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
   351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
   401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
   451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
   501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
   551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
   601 STQTALA
```

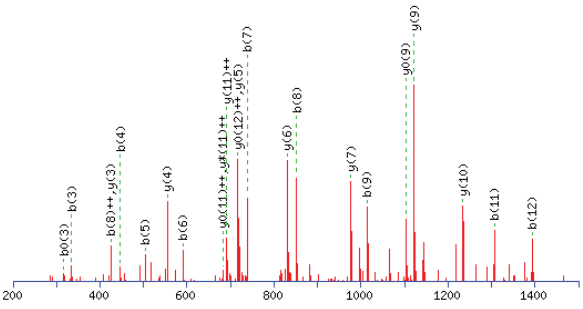
Figure 2. Mascot Report – Protein View.

Peptide View

MS/MS Fragmentation of DAFLGSFLYEYSR
Found in AAN17824, AF542068 NID: - Bos taurus

Match to Query 1338: 1567.823448 from(784.919000,2+)
From data file Z:\Titan_LC-MS\R&D Chemist\SDS Training\SDS for MASCOT\SDS1 Again

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 200 to 1500 Da



Monoisotopic mass of neutral peptide Mr(calc): 1566.74

Figure 3. Mascot Report – Peptide View.

High Resolution Scanning and Extended Mass Range

The 500 Ion Trap high resolution scan option allows the detection of multiply-charged species. The system delivers near baseline resolution on triply-charged ions as indicated below and can easily distinguish charge state five or six. It also extends the mass range of the 500 Ion Trap to *m/z* 3500 Da.

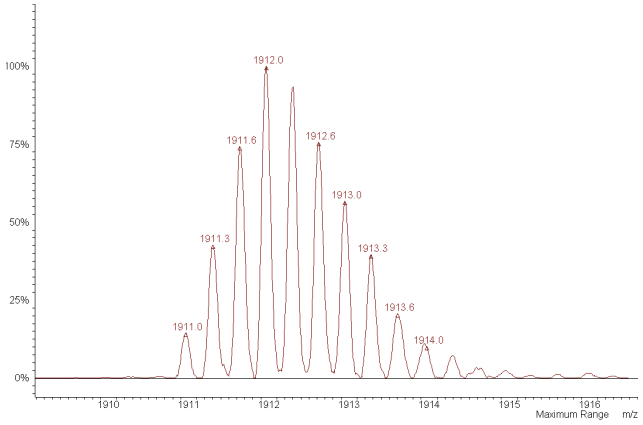


Figure 4. Insulin at +3 charge state, cluster around m/z 1912 is fully resolved. Scanning at 250 Da/sec.

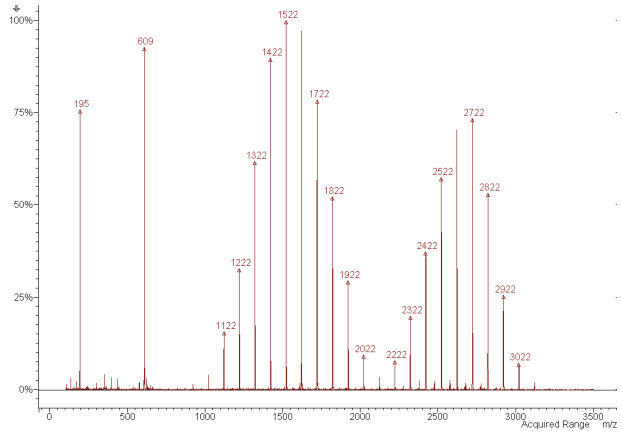


Figure 5. APCI spectrum scanned at 15000 Da/sec of a mixture of analytes containing ions over a wide mass range (< 200 m/z to > 3000 m/z).

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